combinatorial DNA arrays for probing genetic information illustrates the importance of these heterogeneous sequence assays to future science. In most assays, the hybridization of fluorophore-labeled targets to surface-bound probes is monitored by fluorescence microscopy or densitometry. Although fluorescence detection is very sensitive, its use is limited by the expense of the experimental equipment and by background emissions from most common substrates. In addition, the selectivity of labeled oligonucleotide targets for perfectly complementary probes over those with single-base mismatches is poor, preventing the use of surface hybridization tests for detection of single nucleotide polymorphisms. A detection scheme which improved upon the simplicity, sensitivity and selectivity of fluorescent methods could allow the full potential of combinatorial sequence analysis to be realized. The present invention provides such improved detection schemes.

For instance, oligonucleotide-modified gold nanoparticles and unmodified DNA target could be hybridized to oligonucleotide probes attached to a glass substrate in a three-component sandwich assay (see Figures 25A-B). Note that the nanoparticles can either be individual ones (see Figure 25A) or "trees" of nanoparticles (see Figure 25B). The "trees" increase signal sensitivity as compared to the individual nanoparticles, and the hybridized gold nanoparticles "trees" often can be observed with the naked eye as dark areas on the glass substrate. When "trees" are not used, or to amplify the signal produced by the "trees," the hybridized gold nanoparticles can be treated with a silver staining solution. The "trees" accelerate the staining process, making detection of target nucleic acid faster as compared to individual nanoparticles.

The following is a description of one specific system (illustrated in Figure 25A). Capture oligonucleotides (3' - $HS(CH_2)_3$ - A_{10}ATGCTCAACTCT; SEQ ID NO: 43) were immobilized on a glass substrate as described in Example 10. A target oligonucleotide (5' - TACGAGTTGAGAATCCTGAATGCG - 3', SEQ ID NO: 44, concentrations given below in Table 6 for each experiment) was hybridized with the capture oligonucleotides in 0.3 M NaCl, 10 mM phosphate buffer as described in Example 10. The substrate was rinsed twice with the same buffer solution and immersed in a solution containing gold nanoparticle probes

functionalized with target-complementary DNA (5' - $HS(CH_2)_6A_{10}$CGCATTCAGGAT, SEQ ID NO: 45)(preparation described in Example 3) for 12 hours. Next, the substrate was rinsed copiously with 0.3 M $NaNO_3$ to remove Cl^-. The substrate was then developed with silver staining solution (1:1 mixture of Silver Enhancer Solutions A and B, Sigma Chemical Co., # S-5020 and # S-5145) for 3 minutes. Greyscale measurements were made by scanning the substrate on a flatbed scanner (normally used for scanning documents into a computer) linked to a computer loaded with software capable of calculating greyscale measurements (*e.g.*, Adobe Photoshop). The results are presented in Table 6 below.

TABLE 6

Target DNA Concentration	Mean Greyscale	Standard Deviation
10 nM	47.27	2.10
5 nM	53.45	0.94
2 nM	54.56	1.17
1 nM	59.98	1.82
500 pM	61.61	2.26
200 pM	90.06	3.71
100 pM	99.04	2.84
50 pM	135.20	7.49
20 pM	155.39	3.66
None (control)	168.16	10.03

Example 17: Assemblies Containing Quantum Dots

This example describes the immobilization of synthetic single-stranded DNA on semiconductor nanoparticle quantum dots (QDs). Native CdSe/ZnS core/shell QDs (~4 nm) are soluble only in organic media, making direct reaction with alkylthiol-terminated single-stranded DNA difficult. This problem was circumvented by first capping the QDs with 3-mercaptopropionic acid. The carboxylic acid group was then deprotonated with 4-(dimethylamino)pyridine, rendering the particles water soluble, and facilitating reaction of the QDs with either 3'-propylthiol- or 5'-hexylthiol-modified oligonucleotide sequences.

After DNA modification, the particles were separated from unreacted DNA by dialysis. A "linker" DNA strand was then hybridized to surface-bound sequences, generating extended assemblies of nanoparticles. The QD assemblies, which were characterized by TEM, UV/Visible spectroscopy, and fluorescence spectroscopy, could be reversibly assembled by controlling the temperature of the solution. The temperature dependent UV-Vis spectra were obtained for the novel QD assemblies and composite aggregates formed between QDs and gold nanoparticles (~13 nm).

A. General Methods

Nanopure water (18.1 MΩ) prepared using a NANOpure ultrapure water purification system was employed throughout. Fluorescence spectra were obtained using a Perkin Elmer LS 50 B Luminescence Spectrometer. Melting analyses were performed using a HP 8453 diode array spectrophotometer equipped with a HP 9090a Peltier Temperature Controller. Centrifugation was carried out using either an Eppendorf 5415C centrifuge or a Beckman Avanti 30 centrifuge. TEM images were acquired using a Hitachi HF-2000 field emission TEM operating at 200 kV.

B. Preparation Of Oligonucleotide-QD Conjugates

Synthetic methodologies for semiconductor quantum dots (QDs) have improved greatly in recent years, and for some materials, most notably CdSe, monodisperse samples of pre-determined size can now be prepared with relative ease. Murray et al., *J. Am. Chem. Soc.* **1993**, *115*, 8706; Hines, et al., *J. Phys. Chem.* **1996**, *100*, 468. As a result, the unique electronic and luminescent properties of these particles have been studied extensively (see, Alivisatos, *J. Phys. Chem.* **1996**, *100*, 13226, and references therein; Klein et al., *Nature* **1997**, 699; Kuno et al., *J. Chem. Phys.* **1997**, *106*, 9869; Nirmal et al., *Nature* **1996**, *383*, 802), potentially paving the way for QDs to be employed in diverse technologies, such as light-emitting diodes (Schlamp et al., *J. Appl. Phys.* **1997**, *82*, 5837; Dabbousi et al., *Appl. Phys. Lett.* **1995**, *66*, 1316) and as non-radioactive biological labels (Bruchez et al., *Science* **1998**, *281*, 2013; Chan et al., *Science* **1998**, *281*, 2016). However, many applications will require that the particles be arranged spatially on a surface or organized into three-